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Size-exclusion chromatography small-angle X-ray scattering
(SEC-SAXS) of water soluble proteins on a laboratory
instrument
Supporting Information

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Table S1: Details of proteins, SAXS experiments and analysis.

protein ^a	BSA							BSA dimer		RNase A	CAH	HI	OVA	OVA dimer		CA	HAF	
Samples																		
PDB ID	4f5s [1]							4f5s		9rat [2]	1v9e [3]	1ev3 [4]	1ova [5]		1aiv [6]	1ies [7]		
Source ^b	SA							SA		GE	GE	NN ^c	GE		GE	SA		
M_w [kDa] ^d	66	66	66	66	66	66	66	66	133	14	29	35	43	86	76	476	476	
$\epsilon_{280\text{nm}}$ [M ⁻¹ cm ⁻¹] ^e	43824							n/a		9440	50420	5850	31775	n/a	88165	348000		
Buffer ^f	1	1	1	1	1	2	2	3	1	1	1	4	1	1	1	1	1	
c [mg/ml] ^g	7.6	3.7	2.0	1.0	7.6	4.6	4.3	8.1	7.6	7.5	5.4	3.5	4.9	4.9	5.1	11.1	11.1	
Volume [μ l]	500	500	500	500	500	5	25	500	500	500	500	500	500	500	500	500	500	
Amount of protein [mg]	3.8	1.9	1.0	0.5	3.8	0.023	0.11	4.1	3.8	3.8	2.7	1.8	2.5	2.5	2.6	5.6	5.6	
Data collection																		
Instrument ^h	BX	BX	BX	BX	BX	BX	P12	BM29	BX	BX	BX	BX	BX	BX	BX	BX	BX	
Detector ⁱ	P300K	P300K	P300K	P300K	P300K	P300K	P2M	P1M	P300K	P300K	P300K	P300K	P300K	P300K	P300K	P300K	P300K	
Wavelength [\AA]	1.34	1.34	1.34	1.34	1.34	1.34	1.24	0.99	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	1.34	
Flux [ph/s] ^j	$\sim 2.5\text{e}8$	$\sim 6\text{e}7^*$	$\sim 2.5\text{e}8$	$\sim 2.5\text{e}8$	$\sim 4.2\text{e}7$	$\sim 2.5\text{e}8$	$\sim 1\text{e}13$	$\sim 4\text{e}11$	$\sim 2.5\text{e}8$	$\sim 2.5\text{e}8$	$\sim 2.5\text{e}8$	$\sim 2.5\text{e}8$	$\sim 2.5\text{e}8$	$\sim 2.5\text{e}8$	$\sim 2.5\text{e}8$	$\sim 2.5\text{e}8$	$\sim 4.2\text{e}7$	
d [mm] ^k	654	654	654	654	1507	654	3000	2867	654	654	654	654	654	654	654	654	1507	
q_{min} [\AA^{-1}] ^l	0.011	0.011	0.011	0.011	0.0075	0.011	0.0023	0.0054	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.0075	
q_{max} [\AA^{-1}] ^m	0.50	0.50	0.50	0.50	0.22	0.45	0.51	0.48	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.22	
Exposure time [s]	30	30	30	30	30	60	1	1	30	30	30	30	30	30	30	30	30	
Temperature [$^{\circ}$ C]	25	25	25	25	25	25	20	10	25	25	25	25	25	25	25	25	25	
Collection mode	SEC	SEC	SEC	SEC	SEC	static	static	SEC	SEC	SEC	SEC	SEC	SEC	SEC	SEC	SEC	SEC	
Column size & media	10 \times 300 S200 Inc	10 \times 300 S200 Inc	10 \times 300 S200 Inc	10 \times 300 S200 Inc	10 \times 300 S200 Inc	n/a	n/a	10 \times 300 S200 Inc	10 \times 300 S200 Inc	10 \times 300 S200 Inc	10 \times 300 S200 Inc	10 \times 300 S200 Inc	10 \times 300 S200 Inc	10 \times 300 S200 Inc	10 \times 300 S200 Inc	10 \times 300 S200 Inc	10 \times 300 S200 Inc	
Flow rate [ml/min]	0.1	0.1	0.1	0.1	0.1	n/a	n/a	0.7	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
Data analysis																		
# of frames (protein)	12	10	10	10	13	n/a	n/a	40	9	11	10	13	11	10	10	11	11	
# of frames (background)	28 + 12	25 + 20	19 + 5	19 + 10	15 + 20	n/a	n/a	200 + 100	15 + 20	40 + 20	20 + 10	50 + 6	20 + 20	20 + 20	20 + 20	20 + 10	19 + 10	
Data reduction	RAW [8, 9], Python																	
Estimation of q_{eff}	Shanum (ATSAS 2.8) [10]																	
Guinier analysis	AUTORG [11] (individual frames), RAW (averaged data)																	
IFT	BAYESapp [12, 13]																	
Molecular weight determination	SAXSMoW [14]																	
<i>Ab initio</i> modeling	DAMMIF (slow mode), damfilt (ATSAS 2.8) [15, 16]																	
symmetry/anisometry	P1/n.a.	P1/n.a.	P1/n.a.	P1/n.a.	P1/n.a.	P1/n.a.	P1/n.a.	P1/n.a.	P2/prolate	P1/n.a.	P1/n.a.	P3/n.a.	P1/n.a.	P1/oblate	P1/n.a.	P1/n.a.	P1/n.a.	
Computation of crystal structure intensities	CRY SOL (ATSAS 2.8) [17] with background correction enabled																	
Analysis of crystal structure intensities	in-house routine [18]																	
3D representation	PyMol																	
Results																		
Peak c [mg/ml] ⁿ	3.8	1.8	0.9	0.4	3.8	n/a	n/a	n/a	n/a	4.2	2.5	n/a	2.0	n/a	2.6	3.7	3.7	
Shanum q_{eff} [\AA^{-1}]	0.50	0.46	0.46	0.40	0.19	0.33	0.50	0.48	0.50	0.46	0.46	0.49	0.43	0.43	0.50	0.49	0.19	
CRY SOL χ^2	1.08	0.78	0.82	0.80	1.02	1.03	13.34	23.93	1.19	1.17	0.72	1.21	0.75	n/a	1.20	6.50	1.61	
Guinier $I(0)$	4.6e-1	6.8e-2	1.5e-1	6.9e-2	3.6e-3	4.6e-2	4.5e-2	4.9	1.9e-1	1.7e-1	1.6e-1	5.3e-2	1.9e-1	7.0e-2	4.9e-1	2.3e0	1.7e-2	
($\Delta I(0)$) [Arb. Units]	(3.2e-3)	(1.1e-3)	(3.3e-3)	(2.8e-3)	(5.1e-5)	(4.5e-4)	(6.1e-3)	(1.8e-3)	(5.0e-3)	(1.1e-3)	(1.6e-3)	(4.9e-4)	(2.0e-3)	(4.0e-3)	(3.9e-3)	(1.9e-2)	(2.4e-4)	
Guinier R_g	28.3	28.1	27.7	29.0	27.9	28.4	27.7	27.0	41.1	14.9	18.6	20.0	23.5	36.7	30.1	51.6	53.2	
(ΔR_g) [\AA] ^o	(0.3)	(0.6)	(1.0)	(1.7)	(0.6)	(0.4)	(0.1)	(0.1)	(1.4)	(0.2)	(0.3)	(0.3)	(0.4)	(2.9)	(0.4)	(0.6)	(1.1)	
BAYESapp R_g	27.5	28.3	27.1	27.5	27.8	28.4	28.0	27.1	40.8	14.7	17.9	19.1	23.7	36.4	30.4	52.0	52.3	
(ΔR_g) [\AA]	(0.1)	(0.1)	(0.1)	(0.2)	(0.2)	(0.1)	(0.1)	(0.1)	(0.2)	(0.1)	(0.1)	(0.1)	(0.1)	(0.2)	(0.1)	(0.1)	(0.1)	
Crystal R_g [\AA]	27.1	27.1	27.1	27.1	27.1	27.1	27.1	38.7	14.4	14.4	18.3	18.8	22.8	n/a	30.3	53.0	53.0	
BAYESapp D_{max}	79.6	81.0	69.4	73.0	78.9	78.9	79.1	72.6	115.5	41.1	44.2	49.2	65.2	106.1	84.0	119.6	120.4	
(ΔD_{max}) [\AA] ^p	(12.2)	(9.1)	(4.7)	(7.4)	(7.9)	(4.5)	(0.5)	(0.3)	(3.2)	(6.4)	(2.6)	(1.4)	(1.3)	(0.6)	(1.2)	(0.5)	(1.7)	
Crystal D_{max} [\AA]	92.5	92.5	92.5	92.5	92.5	92.5	92.5	92.5	147.5	46.8	58.0	54.9	74.2	n/a	106.1	150.3	150.3	
BAYESapp N_p	4.3	3.4	2.7	2.4	2.4	3.9	7.9	8.6	3.2	3.7	2.7	3.7	3.5	2.8	5.0	6.9	4.4	
(ΔN_p) ^q	(0.2)	(0.3)	(0.3)	(0.3)	(0.5)	(0.2)	(0.2)	(0.4)	(0.6)	(0.6)	(0.1)	(0.1)	(0.5)	(0.3)	(0.5)	(0.2)	(0.5)	
BAYESapp N_s ^r	5.2	5.3	4.5	4.8	4.8	6.0	9.4	11.1	10.6	6.5	3.4	6.1	8.0	6.2	10.4	7.2	7.4	
SAXSMoW M_w [kDa]	65	67	64	61	61	66	66	63	134	10	31	29	40	n/a	76	434	485	

^a Proteins: BSA: bovine serum albumin, RNase A: ribonuclease A, CAH: carbonic anhydrase, HI: human insulin, OVA: ovalbumin, CA: conalbumin, HAF: horse apoferritin^b GE: GE Healthcare, SA: Sigma-Aldrich, NN: Novo Nordisk A/S^c Formulation:Dissolve the protein in water. Add Zn(OAc)₂, phenol, NaCl and sodium phosphate buffer. Adjust concentration to 600 μM HI in 7 mM sodium phosphate (pH 7.4), 60 mM phenol, 200 μM Zn(OAc)₂ and 23 mM NaCl. Check pH of the sample and gently adjust to pH 7.4 using small amounts of HCl or NaOH [19].^d M_w : molecular weight^e $\epsilon_{280\text{nm}}$: extinction coefficient at 280 nm^f Buffers: 1 - PBS, pH 7.4

2 - 50 mM HEPES, pH 7.5

3 - PBS, 1 mM DT'T, pH 7.4

4 - 7 mM sodium phosphate, 60 mM phenol, 200 μM Zn(OAc)₂, 23 mM NaCl, pH 7.4^g c : concentration^h BX: Xenocs BioXolver L (commercial laboratory instrument), P12: BioSAXS beamline P12, EMBL-Hamburg (synchrotron), BM29: BioSAXS beamline ESRF, Grenoble (synchrotron)ⁱ P300K: windowless DECTRIS Pilatus 300K without beamstop, P2M: DECTRIS Pilatus 2M, P1M: DECTRIS Pilatus 1M^j flux at the sample position^k d : sample-detector distance^l q_{min} : smallest measured q ^m q_{max} : largest measured q ⁿ concentration at the maximum of the elution peak^o R_g : radius of gyration^p D_{max} : longest extension of the protein^q N_p : number of good parameters^r N_s : number of Shannon channels^{*} NB: for the measurement on the 4 mg/ml BSA sample, the intensity of the direct beam was lower than for the other measurements.

Contrast calculation

The scattering of a protein sample in the forward direction $I(0)$ is proportional to $(\Delta\rho)^2$, where $\Delta\rho = \rho_{\text{protein}} - \rho_{\text{buffer}}$ is the scattering contrast and ρ_{protein} and ρ_{buffer} are the scattering length densities of the protein and the buffer, respectively. They are given by

$$\rho_{\text{protein}} = \rho_{\text{e,protein}} \cdot r_0 = \rho_{\text{M,protein}} \cdot r_0 / \bar{v} \quad \text{and} \quad \rho_{\text{buffer}} = \rho_{\text{e,buffer}} \cdot r_0 \quad (1)$$

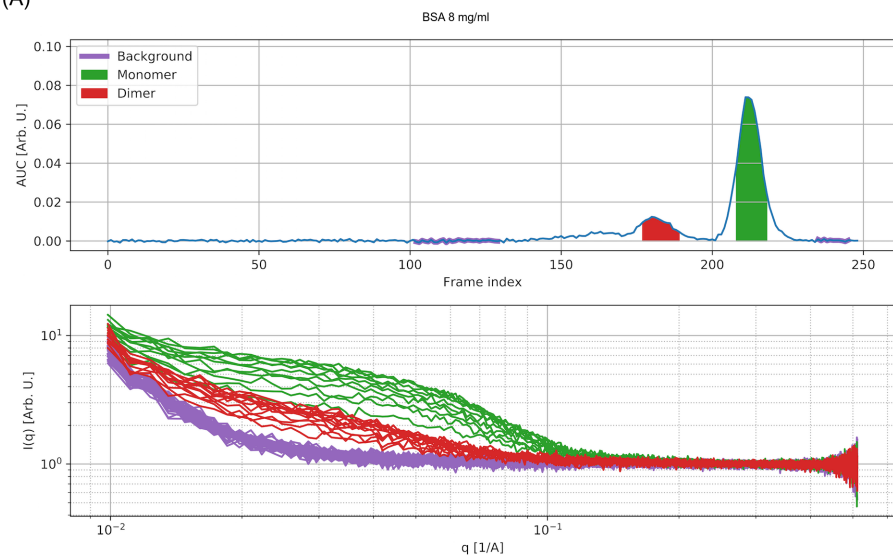
where $r_0 = 2.82\text{e-}13$ cm is the classical electron radius, $\rho_{\text{M,protein}} = 3.22\text{e}23$ e/g [20] is the protein electron density per mass, $\bar{v} = 0.74$ cm³/g is the voluminosity of the protein and $\rho_{\text{e,protein}} = 4.34\text{e}23$ e/cm³ and $\rho_{\text{e,buffer}}$ are the protein and buffer electron densities, respectively. Using the electron densities for PBS buffer, $\rho_{\text{e,PBS}} = 3.37\text{e}23$ e/cm³, and glycerol, $\rho_{\text{e,gly}} = 4.12\text{e}23$ e/cm³, the relative scattering contrast and forward scattering intensity in PBS with glycerol and DTT, respectively, are summarized in the following tables.

$c(\text{gly})$ [%v/v]	$\rho_{\text{e,PBS,gly}}$ [e/cm ³]	$\Delta\rho_{\text{PBS,gly}}$ [e/cm ²]	$\frac{\Delta\rho_{\text{PBS,gly}}}{\Delta\rho_{\text{PBS}}}$ [%]	$\frac{I(0)_{\text{PBS,gly}}}{I(0)_{\text{PBS}}} = \frac{(\Delta\rho_{\text{PBS,gly}})^2}{(\Delta\rho_{\text{PBS}})^2}$ [%]
1	3.38e23	2.70e10	99.2	98.5
2	3.39e23	2.68e10	98.4	96.9
3	3.39e23	2.66e10	97.7	95.4
5	3.41e23	2.62e10	96.1	92.4
7	3.42e23	2.57e10	94.6	89.4
10	3.45e23	2.51e10	92.2	85.1

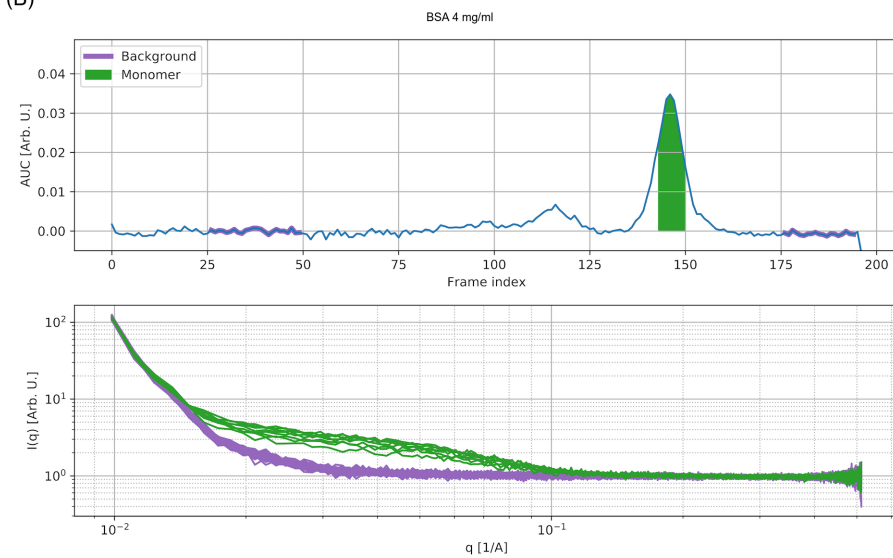
$c(\text{DTT})$ [mM]	$\rho_{\text{e,PBS,DTT}}$ [e/cm ³]	$\Delta\rho_{\text{PBS,DTT}}$ [e/cm ²]	$\frac{\Delta\rho_{\text{PBS,DTT}}}{\Delta\rho_{\text{PBS}}}$ [%]	$\frac{I(0)_{\text{PBS,DTT}}}{I(0)_{\text{PBS}}} = \frac{(\Delta\rho_{\text{PBS,DTT}})^2}{(\Delta\rho_{\text{PBS}})^2}$ [%]
1	3.37e23	2.72e10	99.9	99.9
2	3.37e23	2.72e10	99.9	99.8
3	3.37e23	2.72e10	99.8	99.7
5	3.37e23	2.72e10	99.7	99.5
7	3.37e23	2.71e10	99.6	99.3
10	3.38e23	2.71e10	99.5	99.0

$\Delta\rho_{\text{PBS}}$, $\Delta\rho_{\text{PBS,gly}}$ and $\Delta\rho_{\text{PBS,DTT}}$ are the scattering contrasts of a protein in pure PBS buffer, in PBS buffer with glycerol and in PBS buffer with DTT, respectively, and $I(0)_{\text{PBS}}$, $I(0)_{\text{PBS,gly}}$ and $I(0)_{\text{PBS,DTT}}$ are the corresponding forward scattering intensities.

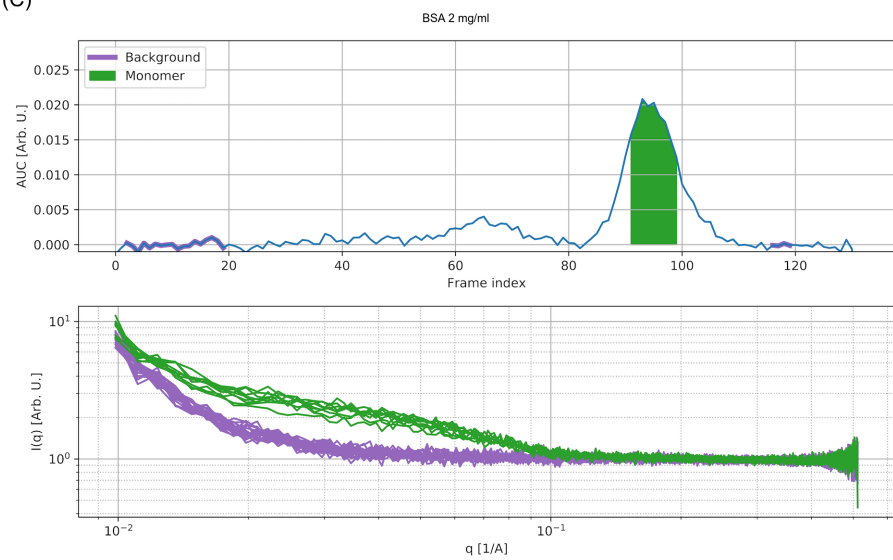
(A)



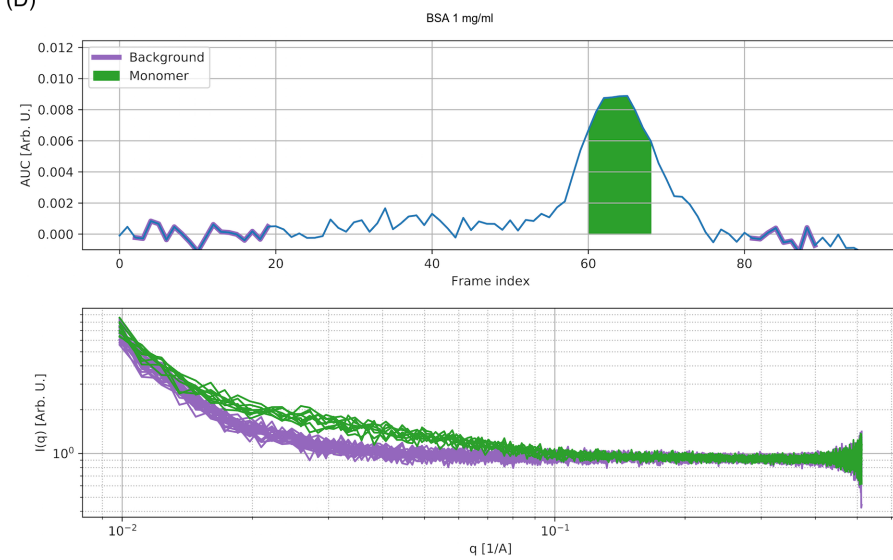
(B)



(C)



(D)



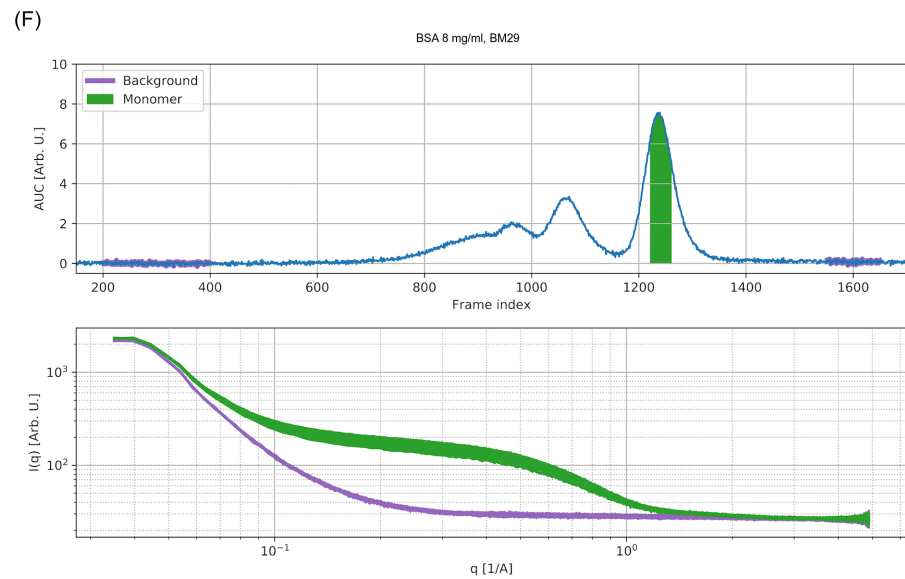
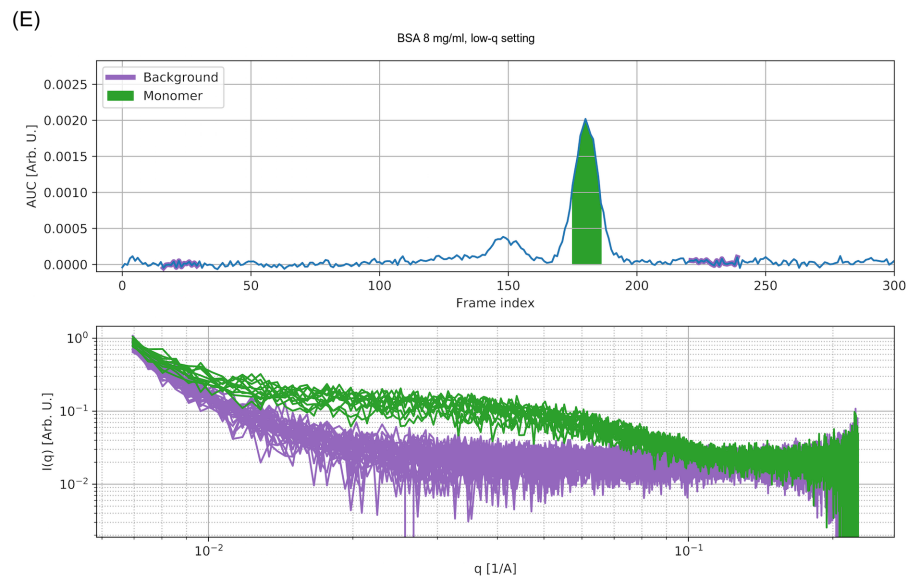
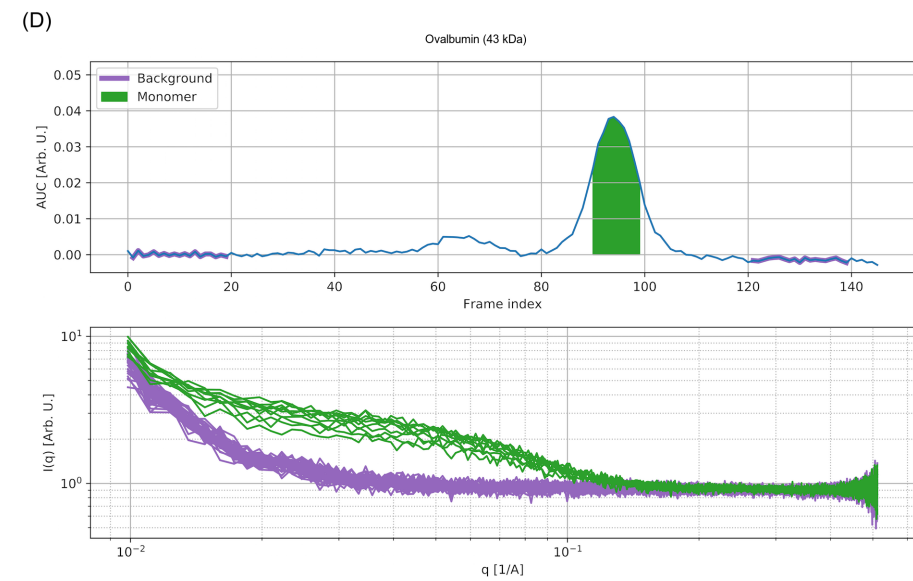
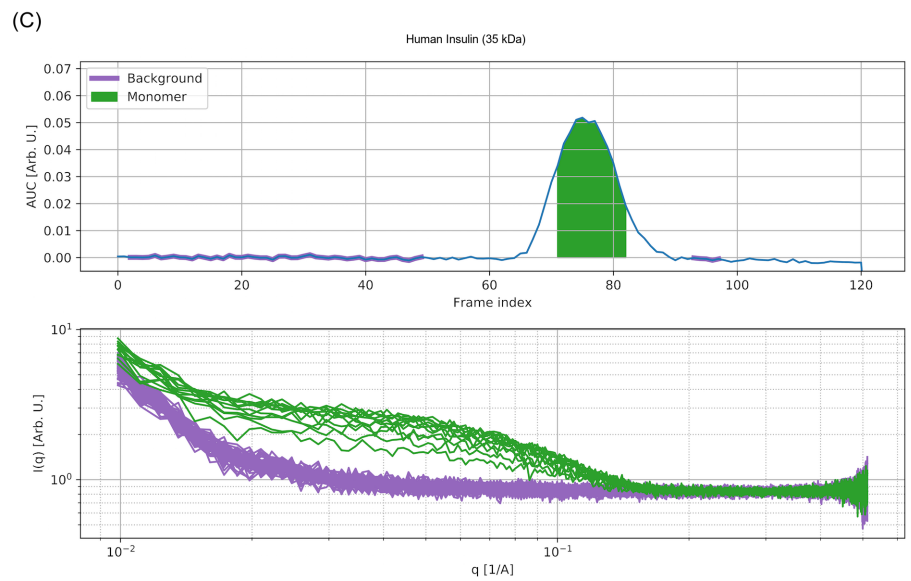
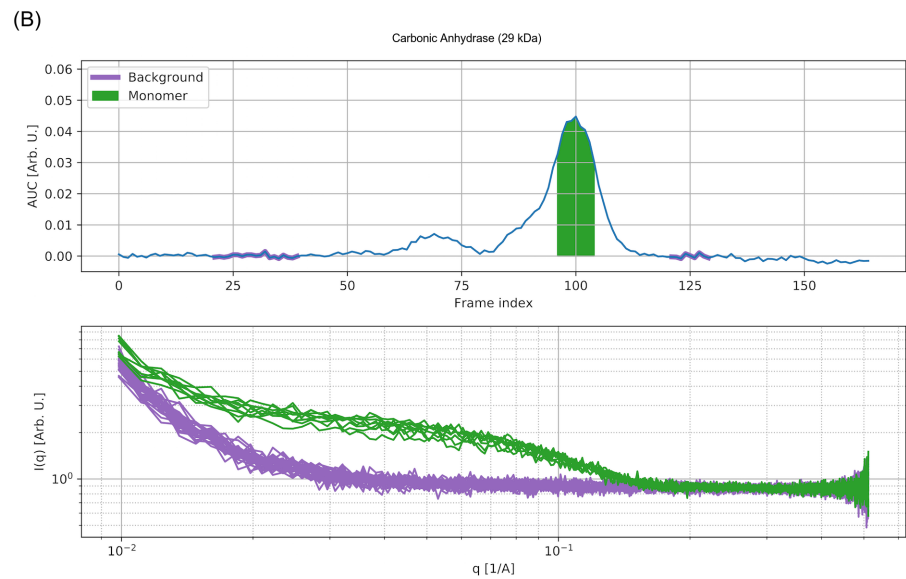
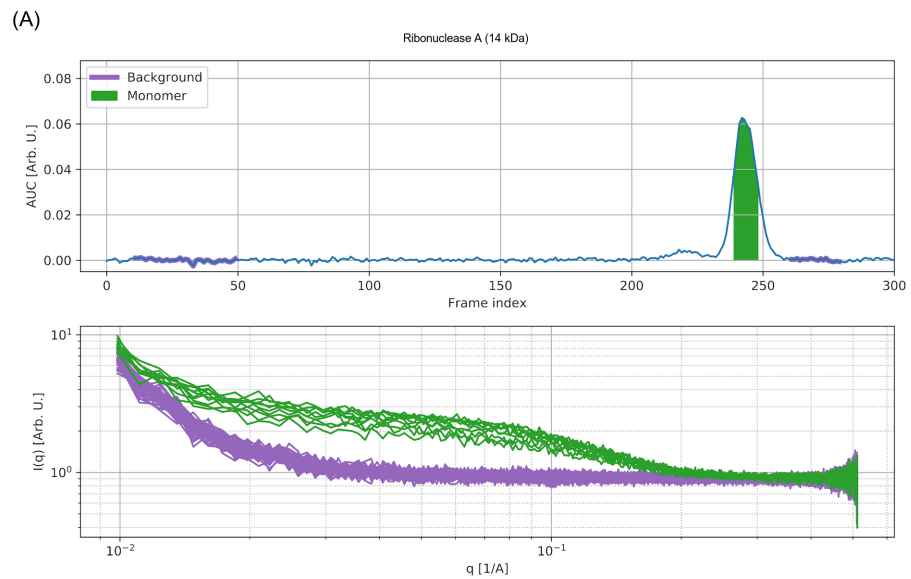


Figure S1: Selection of sample and buffer regions for data analysis of SEC-SAXS data from BSA samples with stock concentrations of 8, 4, 2 and 1 mg/ml. *Top panels:* Integrated intensity as a function of frame index (i.e. time), *bottom panels:* Individual 30s frames. A-E: Xenocs BioXolver L, F: synchrotron BioSAXS beamline BM29, ESRF-Grenoble.



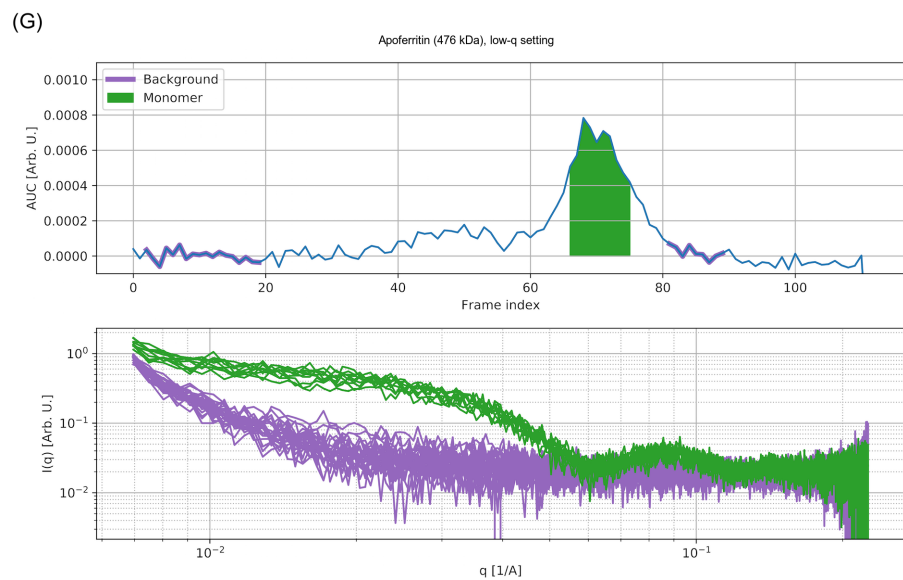
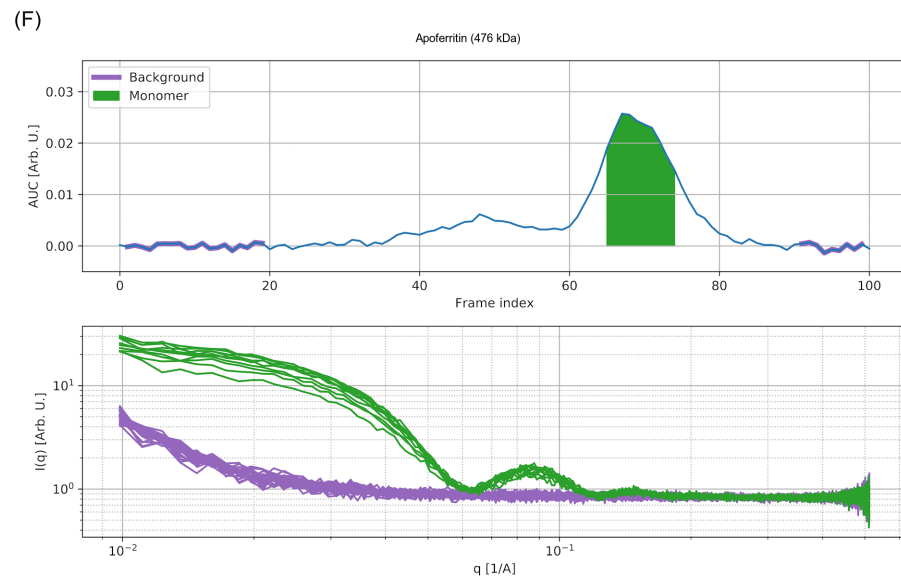
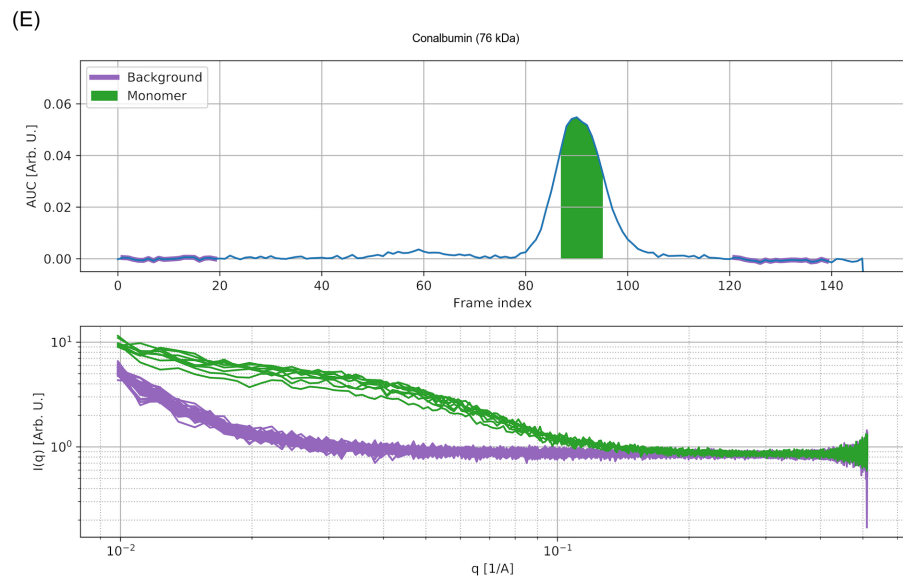


Figure S2: Selection of sample and buffer regions for data analysis of Xenocs BioXolver L SEC-SAXS data. *Top panels*: Integrated intensity as a function of frame index (i.e. time), *bottom panels*: Individual 30s frames.

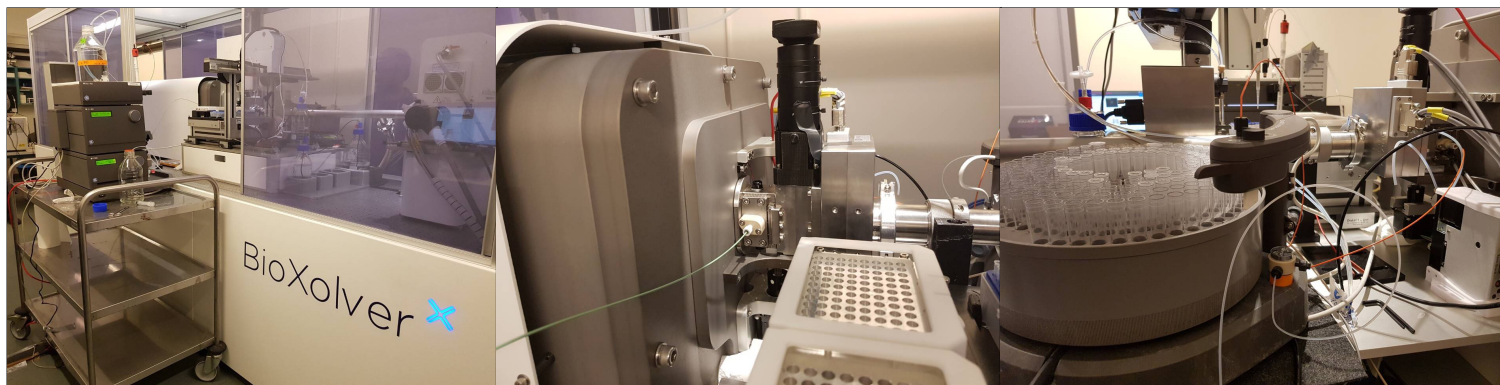


Figure S3: Pictures of our laboratory-based SEC-SAXS setup. Left: Overall view of the mobile HPLC unit next to the SAXS instrument, middle: connection of the HPLC tubing to the flow-through cell, right: fraction collector after the SAXS exposure cell.

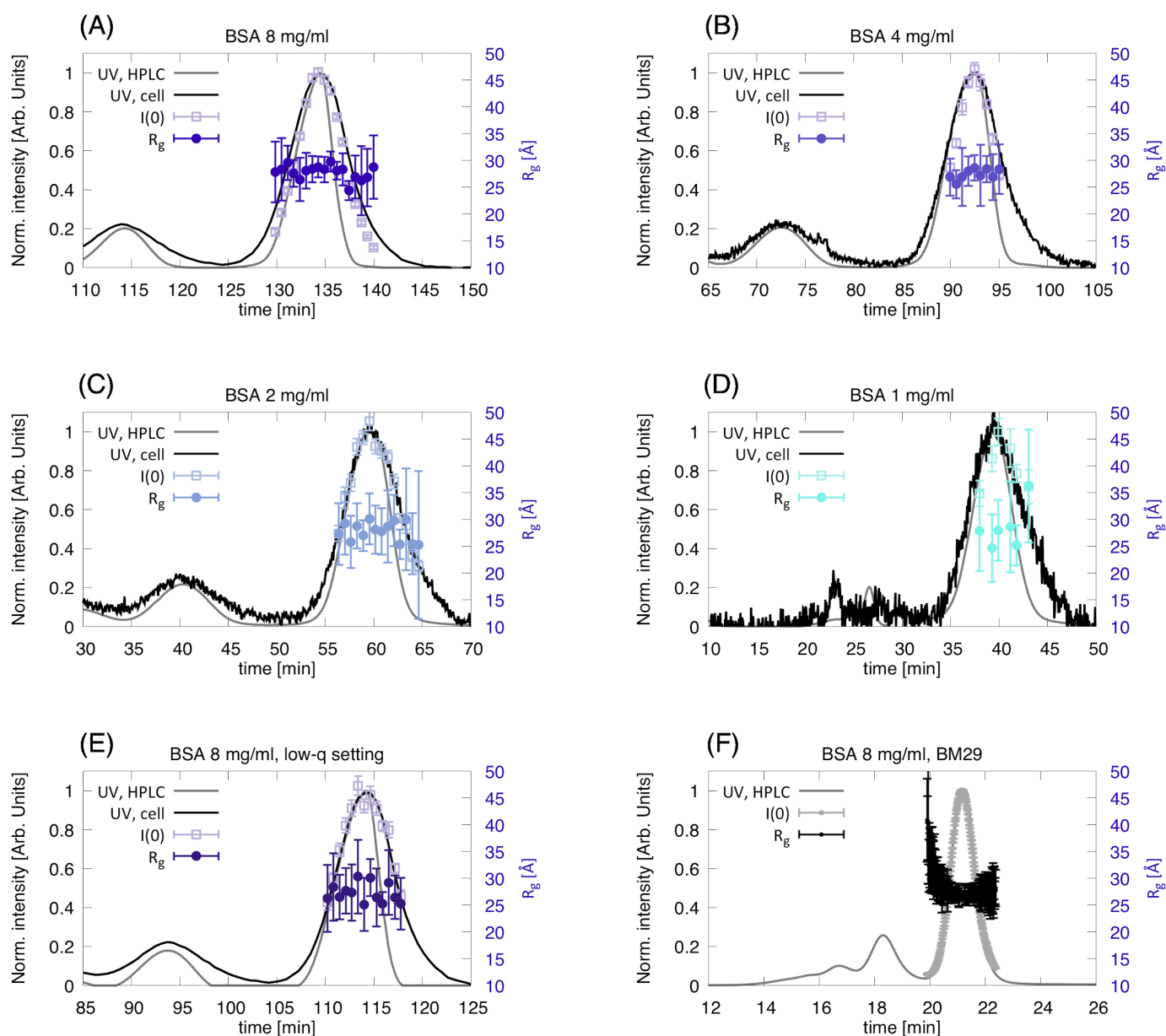


Figure S4: UV traces (HPLC unit and SAXS exposure cell) together with the forward scattering intensity $I(0)$ (left axis) and the radius of gyration R_g (right axis) of each individual frame across the monomer peak of BSA. A-E: Xenocs BioXolver L, F: synchrotron BioSAXS beamline BM29, ESRF-Grenoble.

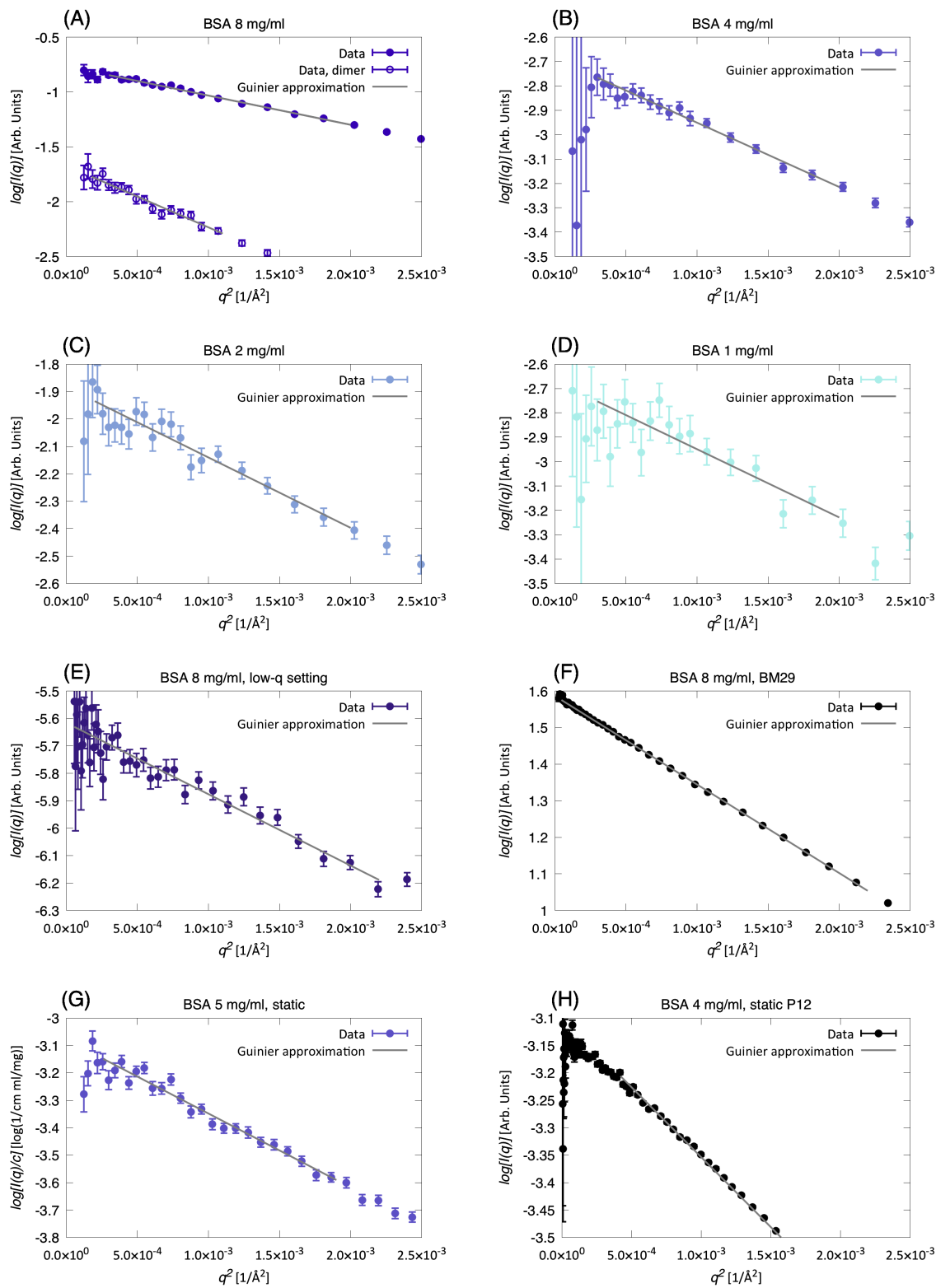


Figure S5: Guinier plots of BSA data. A-F: SEC-SAXS data, G and H: static SAXS data. A-E and G: laboratory instrument (Xenocs BioXolver L), F: synchrotron BioSAXS beamline BM29, ESRF-Grenoble, H: synchrotron BioSAXS beamline P12, EMBL-Hamburg.

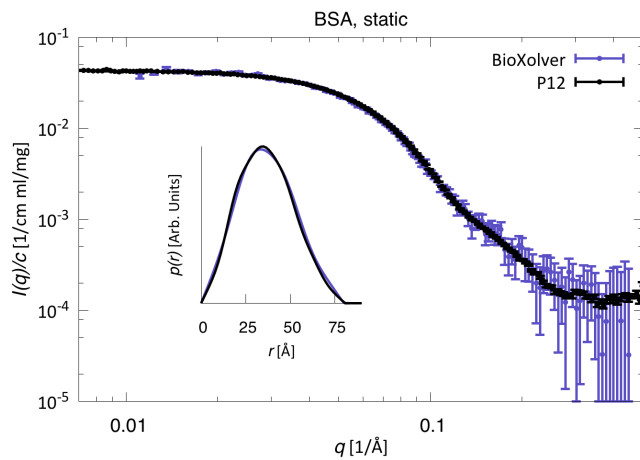


Figure S6: Concentration-normalized static SAXS measurements of 5 mg/ml BSA on our laboratory instrument (Xenocs BioXolver L, 60s exposure) and of 4 mg/ml BSA on a synchrotron BioSAXS beamline (P12, EMBL-Hamburg, 1s exposure) on absolute scale. The synchrotron data was scaled to overlap with the BioXolver data. The inset shows the corresponding pair-distance distribution functions $p(r)$.

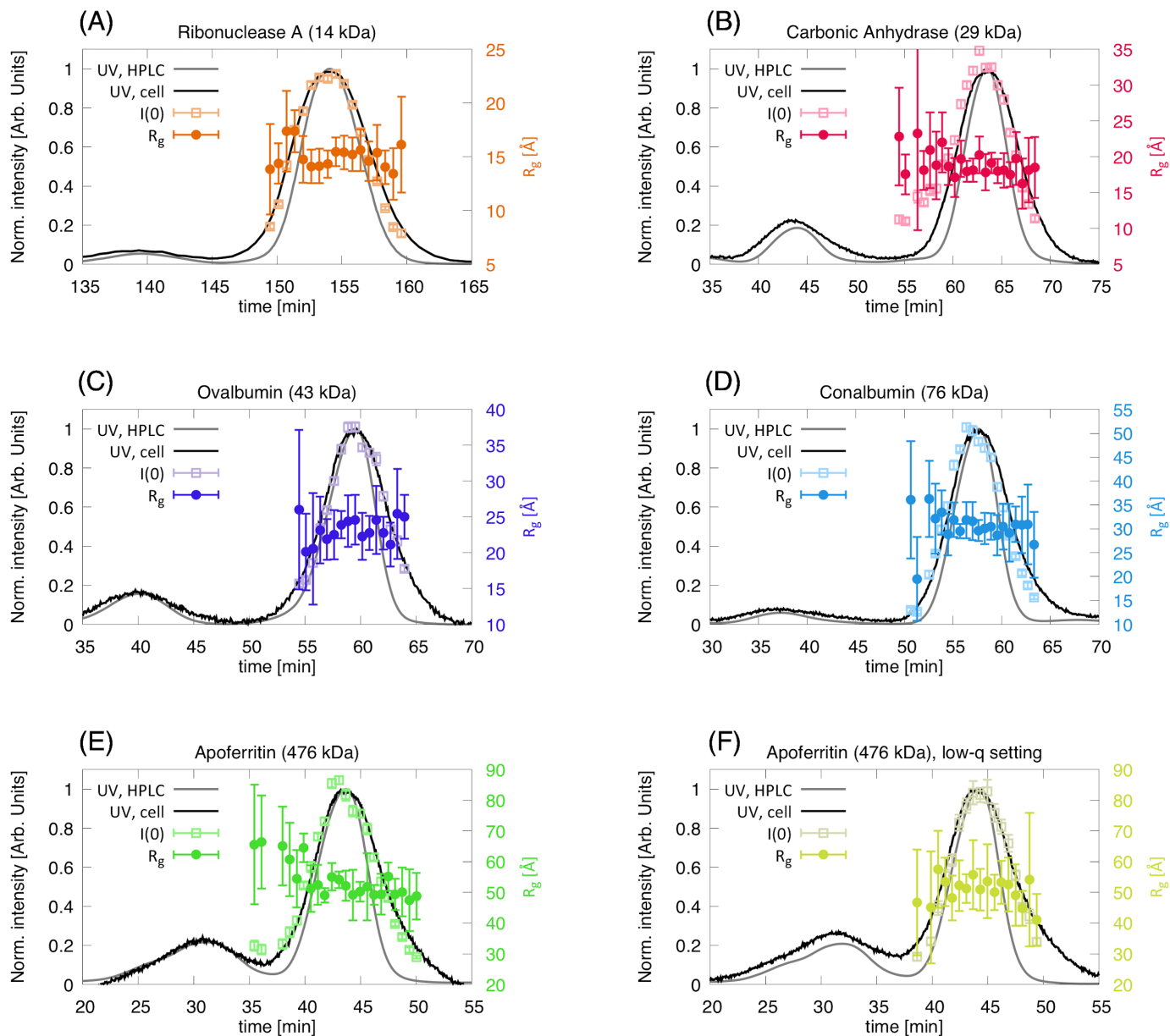


Figure S7: UV traces (HPLC unit and SAXS exposure cell) together with the forward scattering intensity $I(0)$ (left axis) and the radius of gyration R_g (right axis) of each individual frame across the monomer peak of different proteins. *NB:* for HI, no chromatogram is available due to the presence of phenol, which strongly absorbs at 280 nm, in the running buffer.

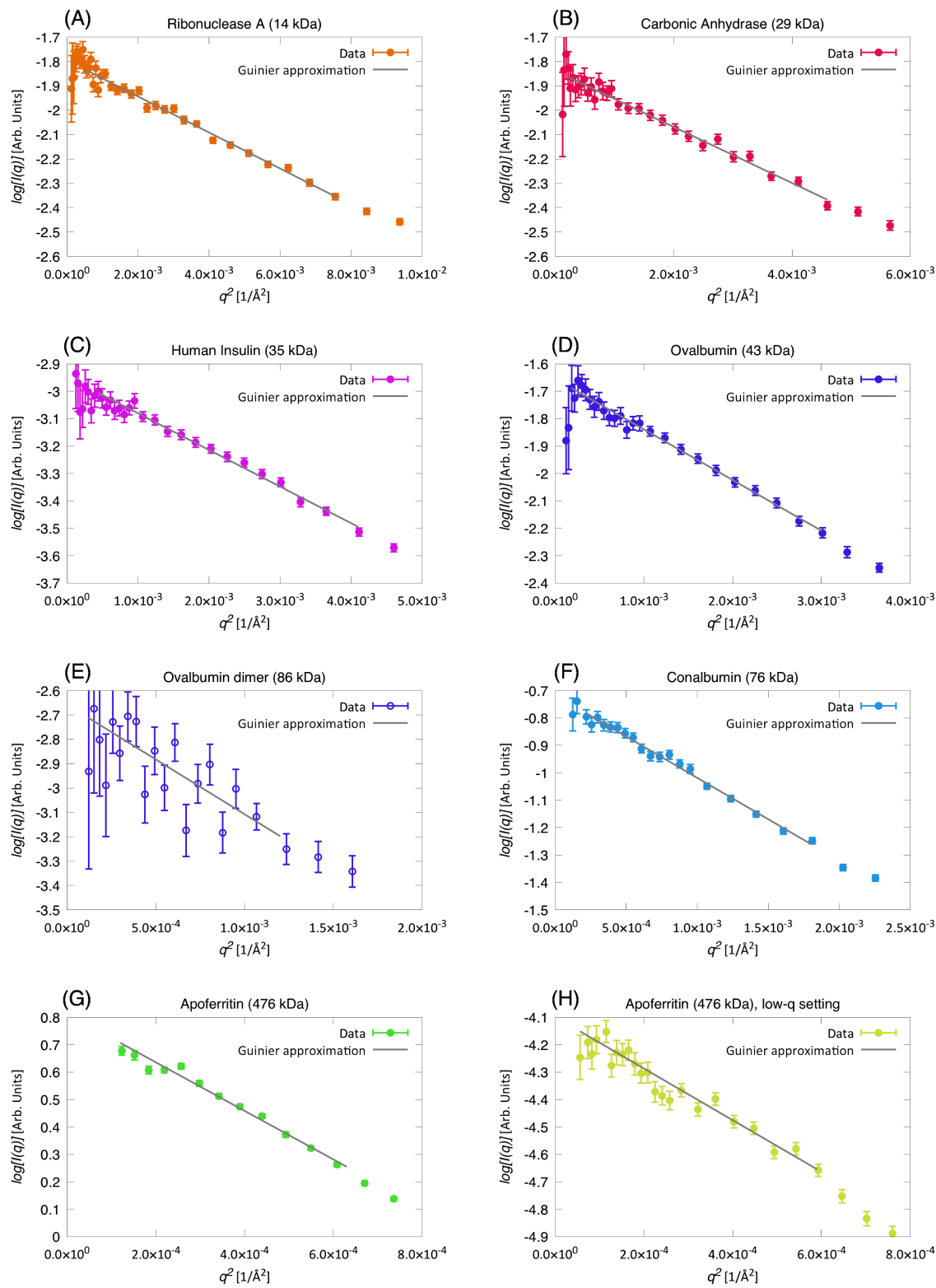


Figure S8: Guinier plots of SEC-SAXS data of ribonuclease A (A), carbonic anhydrase (B), human insulin (C), ovalbumin monomer and dimer (D and E, respectively), conalbumin (F) and apoferritin (G and H).

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